A brief history of iron metabolism

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Summary. A concise history of selected aspects of iron metabolism is presented. According to present understanding, the element is known to be required for transport and reduction of O_2, for reduction of CO_2, N_2 and ribonucleotides, and for other essential cellular processes. The contributions of pioneers in the field, preeminent among them the cell physiologist and biochemist Otto Warburg, are recounted.

Key words. Cell respiration – Cytochromes – Ferritin – Transferrin receptor – Siderophores

Introduction

The present symposium is labelled II since it is the second in a series and follows by five years the first organized by Gunther Winkelmann at Tübingen in 1985. That meeting resulted in the publication of the book 'Iron transport in microbes, plants and animals' (Winkelmann et al. 1987). It has been the hope of those organizing these meetings that the occasion would bring together all workers on iron, be they concerned primarily with microbes, plants or animals. Only partial success has attended these efforts since the sessions have tended to be dominated by the 'microbial' people while the 'animal' and 'plant' workers continue to meet separately on a bi-annual basis, most recently in Australia and Israel, respectively. These latter groups will convene in 1991 in Oxford (England) and Logan (Utah). It is fair to say that the 'animal' people are involved mainly with ferritin and transferrin while the 'plant' contingent is focused on chlorosis. Such division and specialization are probably inevitable with an element having the profound biological importance of iron; nonetheless, we have much to learn from each other and a mixing of the three groups, as is to some degree evident from the attendance at this symposium, should be encouraged.

The present report is a scandalously abbreviated summation of iron metabolism in microbes, plants and animals. Besides the volume already mentioned, the bi-annual reports of the three divisons should be consulted for the most up-to-date information. The book edited by Jacobs and Worwood (1980) is a good general source of knowledge on iron metabolism.

Salient properties of iron and its coordination

Iron is a prominent element on earth and throughout the solar system. The nucleus is built up from argon and is exceedingly stable from the thermodynamic aspect. Among all elements on the surface of planet Earth, it ranks fourth in abundance and is second only to aluminum among the metals. The aqueous chemistry of the two common oxidation states, Fe(II) and Fe(III), is dominated by their solubilities in an environment fixed at neutral pH. The former is quite soluble and one can obtain a 100 mM solution at pH 7 (Hay 1984). The gradual appearance of O_2 in the atmosphere, a process which began about 3×10^9 years ago, in time switched the surface Fe(II) to Fe(III). The solubility product constant of the latter ion may be as small as 10^{-38} M, which would limit the amount of free ferric ion that can be dissolved in water at pH 7.4 to about 10^{-18} M. The general composition of the oxy-hydroxide polymers of ferric ion is FeOOH, which corresponds to an anhydride of $Fe(OH)_3$, although the structure is doubtless more complex (Schwyn 1983).

While there are a number of transition metals that can exist at different oxidation levels, iron seems unique in its capacity to form complexes the redox potentials of which are highly coordination-dependent. Both Fe(II) and Fe(III) prefer to form six-coordinate, octadehedral complexes and the atoms donating electrons to the central metal ion may be O, N or S. With nothing but oxygen around the iron the redox potential will be low, while with only nitrogen in the coordination sphere the potential will be high. This follows from Pearson's rules of 'hard' and 'soft' acids and bases and the electrode equation relating redox potentials to equilibrium constants. A relatively small, highly

charged ion like Fe(III) will be ranked as 'hard' while the larger, less charged Fe(II) will be classified as relatively 'soft'. Since 'hard-hard' and 'soft-soft' make for greater stability, the all-oxygen ligands will preferentially bind Fe(III) while the all-nitrogen ligands have the higher affinity for Fe(II). As a first approximation, we can say that whenever and wherever the higher oxidation state of the metal occurs it must be protected against hydrolysis by some device, such as a cluster of O^- ions or insertion in a special ligand, such as a tetrapyrrole, where the metal ion is no longer free to exchange with the solvent.

Certainly the most common tetrapyrrole for holding iron is isomer IX of protoporphyrin, which bears four methyl, two vinyl and two carboxy-ethyl substituents at the β positions of the pyrrole rings. This is the prosthetic group of hemoglobin and myoglobin, of the hydroperoxidases catalase and peroxidase, and of the b type cytochromes. The reactivity of the coordinated iron is determined by the nature of the substituents carried on the β-positions of the pyrrole nuclei, by the chemical modification of the macrocyclic ring system and by the functional groups that are provided by the protein. The last-named variable is the only one that specifies function in the many proteins using iron protoporphyrin IX as prosthetic group. In cytochrome c oxidase the presence of an aldehyde function shifts the maximum of absorption of the α band into the red, imparting, as Warburg correctly recognized, a green color to the enzyme. Much can be learned about the structural details of these compounds from an examination of their intense and very semantic electronic absorption spectra. Metals besides iron which are inserted in the tetrapyrrole, or some variation thereof, include Mg, Co and Ni. These occur in the chlorophylls, in the vitamin B_{12} series, and in certain methane synthesizers, respectively. The lesson seems to be that Nature, having once discovered an effective ligand, uses it again and again for performance of specific biological tasks.

Iron in hemoglobin

The bright red color of blood attracted scientists through the ages and, guided by the prior work of Sydenham and Willis showing that iron salts could cure anemia in young women, biochemists found early on that the element is a characteristic constituent of blood and is a component of hemoglobin. Over a century ago Zinoffsky purified horse hemoglobin and showed the protein to contain 0.335% iron, a value that suggests the minimum molecular mass.The proof that hemoglobin is an iron protein established the importance of Fe in mammalian nutrition. The story is told well in Underwood's book (1971), which records the original references. Precisely how the iron is coordinated in the hemin molecule, a crystalline product of blood containing nearly all of the iron, had to await the research of Hans Fischer and his school of organic chemists at Munich. His work reached its zenith with the total synthesis of hemin, identical to the coloring matter of hemo-

globin. Fischer was closing in on the correct structure and synthesis of chlorophyll at the time of his death in 1945. He has left a multi-volume account of the chemical synthesis and properties of the common porphyrins (Fischer and Orth 1934, 1937; Fischer and Stern 1940).

Iron in cell respiration

Before Otto Warburg began his research on cell respiration, it was known that hemoglobin carries O_2 to the tissues and that the site of oxidation of foodstuff is in the cells, not in the blood stream. The chemical nature of the respiration enzyme, or *atmungsferment,* remained totally obscure, however, until Warburg performed his famous action spectrum of the CO complex of the enzyme. A valuable biographapy has been prepared by the late Hans Krebs (1981) who did a tour of duty in Berlin as a postdoctoral fellow and who, realizing he was dealing with an unusual personality, kept elaborate records on Warburg. A synopsis of Warburg's research achievements have been summarized by Racker (1982) in a brief review highlighted by the following ditty:

"There was a great scientist named Otto
Who lived by the following motto
'I am always right
My enemies I'll fight
(But I'll be glad to send them my photo)'"

For direct reference the book by Warburg (1949) should be consulted. It was first written in German and shortly thereafter translated into English. This is the ac-

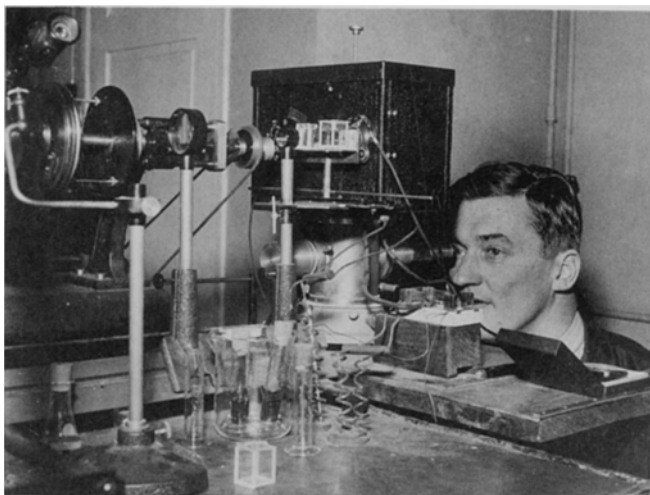Fig. 1. Otto Warburg, reproduced from the English translation of his book (Warburg 1949)

Fig. 2. Hugo Theorell peers into the Warburg spectrophotometer at the Hantverkargatan Laboratory in Stockholm before the instrument and institute were set up in 1948 in new quarters on the Karolinska campus

count that Racker says should be read by 'biochemistry graduate students as an exercise in logical deductions'. For the study of cell respiration Warburg developed a practical manometer that became standard equipment in the biochemical laboratory and which enabled biologists to ascertain which substrates were oxidized and how these compounds might be fitted into metabolic cycles. His father, Emil Warburg, was a noted radiation physicist, and Otto quickly recognized the many virtues of the spectrophotometric technique for assay of enzymes and characterization of coenzymes. The Warburg spectrophotometer was a relatively cumbersome affair, but was considered essential for research with enzymes. Thus in an account of the facilities available at the Medical Nobel Institute in Stockholm, which was inaugurated on May 19, 1948, Hugo Theorell (1948) noted that it contained two rooms for physical measurements, one of which boasted a 'photoelectric spectrophotometer according to Warburg'. The instrument commercialized by Beckman, which was also available in the well-appointed Theorell Institute, was soon to take over the market and has relegated the respirometer to the Museum of Scientific Apparatus (Fig. 2).

To return for a moment to Warburg's action spectrum of the CO complex of cytochrome c oxidase, as the enzyme came to be known. This is widely acclaimed as probably the most elegant experiment yet performed in biochemistry. Warburg knew of the previous work of J. S. Haldane on the photochemical dissociation of carboxy hemoglobin, so that when he measured a spectrum with prominent α, β and γ (Soret) bands, he concluded that he must be dealing with an iron porphyrin. This was done with light of only a limited number of wavelengths and without isolating the pure enzyme. Warburg complained in his Nobel lecture that the *atmungsferment* was as 'unavailable as the material of the stars', although he did prepare several dehydrogenases in crystalline form. Now we know that cytochrome c
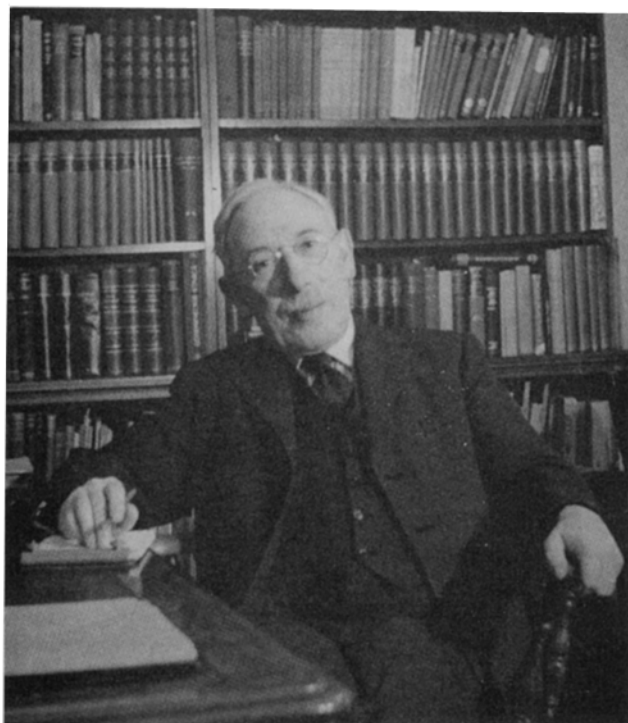

Fig. 3. David Keilin, reproduced from the posthumously published account of his work on cell respiration (Keilin 1966)

oxidase is a complex membrane protein consisting of several subunits and, like many enzymes reacting with molecular oxygen, contains copper (Capaldi 1990). Prodigious though his contributions may have been, Warburg's effectiveness was compromised by a certain arrogance and by a fanaticism that confounded and aliented his colleagues. These attributes have been enumerated by Krebs (1981).

At this juncture a Polish entomologist working in Cambridge, David Keilin, recognized that the pigments in insect wings were connected to cell respiration and he named the colored components cytochromes. Armed with nothing much more sophisticated than a spectroscope, he deduced correctly the order of reactivity and role of cytochromes in cell respiration. Following his death in 1963 his daughter, Joan Keilin, completed the manuscript then in preparation and published the material (Keilin 1966) (Fig. 3).

Diverse roles of iron in biology

Today we recognize that iron enjoys a quite unique niche as a trace element. Although a true trace element, it is generally required at a substantially higher level than other elements of the transition series. The human requirement of the mineral is both age and sex determinant; an uptake of about 1 mg/day suffices to keep the body in a state of homeostasis. The total amount that must be consumed in the diet is guessed to be about ten times this, or 10 mg. The iron content of the legendary 70-kg man is estimated to be about 5 g, which is the

mass of a no. 8 common nail. If we assume a hemoglobin content of 16% and a blood volume of 8 l, it can be readily calculated that the body contains about 1280 g of this specific hemoprotein. At 0.34% iron, this comes to 4.35 g of the metal. Virtually all of the remaining iron is in the storage protein, ferritin, with only microscopic amounts left for the myriad of iron-containing enzymes. Since we have $4350/56 = 78$ mmol iron present as hemoglobin, a poison such as cyanide must react by some mechanism other than by binding to this protein. As Warburg showed, cyanide forms a complex with the oxidized state of cytochrome oxidase, and this is the reason why the lethal dose of the poison is only about a millimole.

It has not been possible to demonstrate that life can exist in the absence of iron. The best candidates are certain lactobacilli which have evolved to live in dairy products which are notoriously low in iron and high in lactoferrin, a glycoprotein that complexes Fe(III) in a form that is unavilable to many microorganisms. Lactics grow at maximum rates on media used to propagate yeast, and cells of the bacteria collected from low iron media contain only a few atoms of iron (Archibald 1983). This is thought to be contaminating iron since the true lactics are totally lacking in peroxidases, catalases and cytochromes – indeed they have no heme compounds whatsoever. While they could conceivably arrange their energy metabolism in such a way as to avoid use of iron enzymes, there would seem to be no means whereby they could obviate the use of ribonucleotide reductase as a mechanism for synthesis of DNA. Here we find that Nature has equipped them with the vitamin B_{12}, cobalt-containing reductase for generation of the deoxynucleotide precursors of DNA.

Among the other significant roles of iron in biology we should mention nitrogenase, the enzyme complex reducing atmospheric nitrogen to ammonia. Although this enzyme is found only in the bacterial world, it makes possible both prokaryotic and eukaryotic life forms. Typically nitrogenase contains either molybdenum or vanadium, together with iron, but some forms of the enzyme contain only iron. The number of bacterial species capable of fixing N_2 is quite impressive and includes both aerobes and anaerobes, but eukaryotic microbes such as yeasts and fungi appear to lack the enzyme.

Iron is required for synthesis of the photosynthetic apparatus and is hence of vital economic importance since crop yields are correlated directly with capacity to fix CO_2. Plant iron deficiency, commonly referred to as chlorosis, is manifested by yellow leaves and stunted growth. The condition occurs in virtually every country on the globe, but is endemic, for example, in the dry, calcareous soils of the inter-mountain region of the United States. Chlorosis can be abated through cultivation of plants which have an enhanced capacity to use iron, which is generally present but unavailable, or via application of chelating agents. The latter method is expensive, however, and it is considered urgently necessary to find an effective means of combating chlorosis. Exactly how the lack of iron impacts chlorophyll syn-

thesis is still under study, but may be related to the synthesis of δ-aminolevulinate, a precursor of the tetrapyrrole ring of chlorophyll, which comes out of the tricarboxylic acid cycle. Aconitase is an iron enzyme. Ferredoxin, a low-molecular-mass protein rich in iron and required for both N_2 and CO_2 fixation, may also be in low supply when iron is limiting (Miller et al. 1984).

Every year brings forth new reports on the ubiquitous iron-sulfur proteins, some of which apparently operative via a non-redox function. The enzyme already mentioned, ribonucleotide reductase, when isolated from Escherichia coli and man contains iron, but from other sources the metal may be manganese or cobalt. The tartrate-resistant acid phosphatases of mammalian tissues appear to carry two ferric ions oxo-bridged in a complex imparting a pink or purple color (Vincent and Averill 1990).

Iron assimilation is membrane-regulated

There seems to be no known biological mechanism for the excretion of iron, uptake of the element being regulated at the membrane level in all species studied. The toxicity of iron (see below), only recently appreciated, gives a good biological rationale for this mode of regulation. The pioneering work in this field was done by McCance and Widdowson in 1937 who applied radioiron to demonstrate that iron excretion is a very limited process. A special, highly efficent mechanism for uptake of iron as heme apparently exists in the human. The year 1937 was a banner one for mammalian iron metabolism for that was when Laufberger isolated ferritin in a crystalline state. Remarkably, the stimulation of ferritin synthesis at high iron and the enhancement of transferrin receptor synthesis at low iron appears to be mediated at the mRNA level and by effectors which interact with either upstream or downstream iron responsive elements (IREs). This aspect of regulation is dealt with elsewhere in this symposium and in the review by Worwood (1989).

In 1973 Lankford wrote a review on bacterial iron assimilation that remains the 'primary' standard in its field. He coined the term 'siderophore' to designate the family of low-molecular-mass, virtually ferric-specific ligands elaborated by microorganisms to combat iron deficiency (Lankford 1937). These are generally 'hard' base ligands with a preponderance of oxygen atoms disposed in space to chelate effectively with Fe(III). Many of these ligands are catechols or hydroxamic acids, but a third type, which contains neither of these ligand systems, is exemplified by rhizobactin (Smith et al. 1985) and staphyloferrin (Konetschny-Rapp et al. 1990).

The synthesis of both catechol- and hydroxamate-type siderophores is achieved by E. coli, e.g. enterobactin and aerobactin. In every case the siderophore and its transport are regulated by a repressor protein, Fur (ferric uptake regulation) that reacts, as its Fe(II) complex, with an operator sequence consisting of a 19-bp palindromic 'iron box' array: 5'GATAATGATAAT-

CATTATC (Bagg and Neilands 1987). Mutants lacking the Fur repressor hyperexcrete siderophores, but are affected in many additional functions and hence the high-affinity iron-uptake pathway is part of a complex regulatory circuit.

Iron and infection

The human body has evolved elaborate defenses to deprive an invading microbe of iron while the pathogen, in turn, has developed specialized mechanisms to acquire the element in a hostile environment. The several scholarly reviews on the topic by Weinberg (1984) are highly recommended and a book on the subject has recently appeared (Bullen and Griffiths 1987). The virulence of *E. coli* appears to be enhanced substantially by the capacity to synthesize aerobactin. This may seem odd since the common siderophore made by enteric bacteria, enterobacin, has the higher affinity for Fe(III), even at neutral pH. However, the affinity of aerobactin for Fe(III) is such that it can leach iron from transferrin and, as a non-aromatic ligand, it does not get tangled in the proteins as does enterobactin.

While siderophores have been implicated in iron acquisition by various microbes, we must hasten to add that there is no evidence, even by the super-sensitive CAS assay, that some species form chelators. The list includes, among the bacteria, *Hemophilus, Neisseria,* strict anaerobes and the lactics, and among the fungi, *Saccharomyces cerevisiae* and *S. pombe.* Some of these appear to obtain iron by reduction at the surface while others, such as *N. gonorrhoeae,* have discovered how to make receptors which extract the iron from iron transferrin (Blanton et al. 1990).

The important role of iron in infection was placed on a logical footing by the seminal research of Schade and Caroline (1944) who found that the anti-bacterial activity of egg white could be reversed by addition of iron salts. They eventually purified an iron binding protein from blood and labelled it 'siderophilin' (Schade and Caroline 1946). At about the same time Laurell and co-workers in Sweden independently isolated an iron-containing protein from the same source and designated it 'transferrin'. The three glycoproteins, namely, serum transferrin, ovotransferrin and lactoferrin, appear to have the function of keeping iron away from bacteria while making the element available to host tissues.

Fertilizing the ocean with iron

There has lately been some talk about adding iron to the ocean in order to stimulate algal growth and so remove excess CO_2 from the atmosphere. True, the airborne CO_2 is rising at an alarming rate. It is up about 25% since the dawn of the industrial age at about the middle of the last century. The recent work on the fertility of waters in the Antarctic suggests that iron is the limiting nutrient (Martin et al. 1990). But before fertilizing the oceans of the world with iron salts, the 'Geritol' solution to greenhouse gases, we should recall that the ocean is an ecosystem established over eons of time. Intervention by provision of an element with the multiple and fundamental biological properties of iron may lead to untoward results in the future. Thus redox-active metal ions like iron and copper can act catalytically to convert partially reduced oxygen species to the potent oxidizing agent, hydroxy radical. The relevant chemistry, still not completely understood, may be as follows:

$$O_2^- + M^{higher} = O_2 + M^{lower}$$
$$H_2O_2 + M^{lower} = OH^- + OH^\cdot + M^{higher}$$
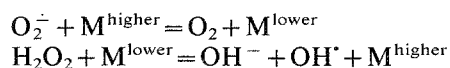
where M is the regenerated metal ion.

A better idea would be to attack the problem at its source, the profligate consumption of fossil fuels. According to a recent report issued by Greenpeace News, the United States consumes 17 billion barrels of oil per day, almost half of which is imported; with 5% of the world's population, it operate 35% of the automobiles, a major consumer of petroleum. The article points out that over the past decade federal support for public mass transit and alternative energy has been cut by 50% and 90%, respectively. Thus conservation and solar development would seem to be superior methods over tampering with the composition of the ocean for reduction of CO_2 emissions.

The future

Our knowledge of iron metabolism in microbes, plants and animals, although impressive, still has far to go to be understood fully. It would be gratifying if research on this topic could be supported as a purely intellectual endeavor, not connected directly to the exclusive welfare of the human animal. Scientific research is the cornerstone of the industrialized state, a way of life that obviously must now be replaced by a more bio-centric society. These philosophical thoughts should perhaps be borne in mind as we ponder the progress that may be made in all spheres of research in the ensuing five years.

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